Caffine Protocols 1/12/79

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Protocol No.: 1150

November 14, 1978

FDA PROJECT PROTOCOL

TERATOGENIC POTENTIAL OF CAFFEINE IN OSBORNE-MENDEL RATS (ADMINISTERED VIA ORAL INTUBATION)

Objective: Caffeine, a naturally occurring substance and a food additive when added to soft drinks, is one of the compounds which is known to cross the placental barrier. It has been studied in numerous teratology and reproduction studies utilizing various modes of administration, numerous species and strains of animals, and many regimens. A full review of these articles is found in a memorandum dated August 8, 1978. Due to deficiencies that exist within these various studies and due to the fact that many were conducted in the 1960's and early 1970's, it was thought that studies should be run to attempt to elucidate whether caffeine is a teratogen and if so, to further resolve the no-effect level. These protocols will not deal with the teratological studies done by intraperitoneal and subcutaneous dosing, since they are of little relevance to the Bureau of Foods and have very limited usage, nor will they address the question of dietary feed since little caffeine is consumed in food.

Proposed Starting Date: Depending upon supply of animals and availability of adequate personnel and equipment.

Proposed Ending Date: Animals will be treated 20 days during pregnancy but it is impossible to determine how quickly the animals will mate.

Name of Study Director: Thomas F.X. Collins, Ph.D.

Other Principal Investigators Involved: Thomas Black, M.S., John Welsh, Ph.D.

Technicians Involved: George Gray, John Goodman, James Rorie

Animal Care Personnel: John Goodman, James Rorie, Michael Scott

Skeletons Read By: John Welsh, Ph.D.

Wilson Sections By: Thomas Black, M.S.

Testing Facility: Mammalian Reproduction and Teratology Unit
Whole Animal Toxicology Branch
Division of Toxicology
FDA

Room to be Utilized: Rooms 5452 and 5454

Compound Information: Caffeine, the alkaloid 1,3,7-trimethylxanthine, was purchased from the Eastman Kodak Company (lot B7X) and has been submitted to the Analytical Chemistry and Physics Branch for a thorough chemical analysis and stability determination. Preliminary indications are that the samples are approximately 99% pure.

The estimated caffeine consumed from cola beverages over a 14-day period has been shown to vary from 5.5 to 16.0 mg/kg/day. In humans, caffeine appears to equilibrate freely between plasma and tissue water; it has been shown to cross the placental barrier. Ingested caffeine is absorbed, metabolized and excreted in the urine as xanthine derivatives.

Animals and Animal Husbandry Procedures: Osborne-Mendel rats (FDA strain) are obtained from the FDA breeding colony. The females are between 100 and 140 days of age and weigh between 220 and 270 grams when placed on the experiment. The males are sexually mature animals approximately 5-6 months of age.
168 males are utilized as sires in this experiment. Fifty pregnant females per dose level are utilized.

The animals are housed in hanging cages. The animals are mated in double hanging cages and the females are housed in single hanging cages. Animals are provided with ground Purina Chow. The animal pans are changed every Monday, Wednesday, and Friday except the mating racks which are changed daily to aid in the identification of vaginal plugs. Floors are mopped daily and there are at least two checks daily for morbid animals.

Since the animals are given the compound over the entire period of pregnancy, this necessitates dosing to occur on weekends. Appropriate professional and technical staff are available to work during weekend work periods. The animals are uniquely identified by metal ear tags.

Methodology: The compound will be given by oral intubation at dose levels of 0, 6, 12, 40, 80, and 125 mg/kg/day on days 0-19 of gestation. A matched set of stainless steel gavage needles (16-gauge X 3 inches with stainless steel ball tip) will be used. Caffeine will be dissolved in distilled water. The solution will be made up daily and given at the same time each day. Controls will be intubated with distilled water. The compound is weighed on an analytical balance which is calibrated before each weighing by utilizing the scale calibration form (F-10) and the standard operating procedure for the calibration form (SOP-10). The solutions are made up in clean volumetric flasks. The study follows the guidelines established for gavage studies and utilized in the FD&C Red No. 2 Collaborative Study.

The mating procedure is described in SOP-1 and records of all animals mated are kept on mating forms (F-1). Each evening at approximately 4:30 pm, 72 females which meet the requirements (i.e., weight between 220 and 270 g and 100-140 days of age) are mated with 36 males which are randomly chosen from the male population available. The following morning, the females are removed, the females are individually smeared for the determination of sperm in the vaginal fluid. This is examined under a binocular microscope. The females exhibiting a positive sign of pregnancy are randomly (utilizing a table of random numbers) placed on the experiment. The date of finding sperm is considered day 0 of pregnancy. Each female is placed in an individual cage and given a known amount of ground chow and a water bottle containing distilled water or the appropriate amount of compound in solution. Each water bottle contains a sipper tube with a metal ball to prevent extraneous loss of fluid. The amount of food given and consumed is recorded on the food consumption form (F-5).

All data pertaining to experimental assignment are recorded on the appropriate form (F-2) according to the standard operating procedure for this form (SOP-2). The mating procedure continues until there are 50 females per dose level.

The animals are weighed on days 0, 7, 14, and 20, and food consumption is measured on the same days. For these records, the food consumption form (F-5) is used.

On day 20, the animals are sacrificed by carbon dioxide asphyxiation and a cesarean section is performed according to the procedures described in SOP-4. The following parameters are noted. The number and position of resorption sites and fetuses (dead or alive) are recorded, as well as corpora lutea data and number of implantation sites. Fetuses are examined individually. Fetus sex and number of normal and abnormal fetuses are recorded. Records of cesarean section data are made on forms F-4. Color photographs are taken on viable fetuses of the negative control or test substance group exhibiting grossly apparent anomalies. One-half of the fetuses, randomly selected, are submitted to visceral examination by Wilson technique and the remaining half of the fetuses are submitted to skeletal examination by Alizarin technique. The randomization, however, is not done on fetuses with grossly apparent anomalies; these are assigned to that method which will better define the anomaly.

The skeletons are stained according to the technique described in SOP-11 and the documentation of the staining is recorded on the appropriate stain documentation form (F-11). The skeletal analysis is based on procedures described in SOP-8 and also from skeletal atlases available in the laboratory.

Wilson section data are recorded on the appropriate Wilson section forms (F-9). The procedures are based on the methodology developed by James Wilson (see SOP-9). Also utilized are several manuals, such as that developed by Jackson and one in our own laboratory. Photographs of various anomalies are also available in the laboratory.

Data Reporting: Data reported will include, but not be limited to, the following elements:

Chemical tested (or control)
Species identification
Dose level
Dam identification
Dam weights (at 0, 7, 14, and 20 days)
Food consumption (at 0, 7, 14, and 20 days)
Fate of dam (term, died, aborted) and date
Date dam sectioned
Number and positions of implantations
Number and positions of resorptions
Number, weight, length, and position of live fetuses
Number, weight, and position of dead fetuses
Pup identification
Abnormalities for each pup
Sex of each pup

Analysis of Data: All the data will be doubly validated and sent to the FDA's Division of Mathematics for statistical analysis. The specific analyses performed will be reported in the manuscript prepared from the data.

The data analysis methods are usually similar to those described in Food and Cosmetic Toxicology 10: 619-624, but may vary slightly depending on the latest information available on specific tests.

Thomas F.X. Collins 1/4/79
Thomas F.X. Collins Date
Study Director

Frank Vocci Jan 79
Frank Vocci Date
Chief, Whole Animal Toxicology Branch

W. Gary Flamm 1/2/79
W. Gary Flamm Date
Associate Director for Regulatory Evaluation

Eugene M Sporn 1/4/79
Eugene Sporn Date
Associate Director for Laboratory Investigations

James L. Winbush 12/27/78
James Winbush Date
Chief, Division of Mathematics

Protocol No.: 1150 January 12, 1979

FDA PROJECT PROTOCOL

TERATOGENIC POTENTIAL OF CAFFEINE IN OSBORNE-MENDEL RATS (ADMINISTERED VIA ORAL INTUBATION).
ADDENDUM NO. 1

Proposed Starting Date: The study is expected to start January 15, 1979.

Proposed Ending Date: It is impossible to assign an exact ending date since it depends upon how soon the animals will mate.

Compound Information: An analysis of caffeine to be used (Eastman Kodak Company lot B7X) has been received from the Eastman Kodak Company and it appears to be at least 98% pure and greater than USP specifications. Since the caffeine solution is being made up fresh every day, no stability data is required.

Thomas F.X. Collins 1/12/79

Thomas F.X. Collins, Ph.D. Date
Study Director

Protocol No.: 1151 November 14, 1978

FDA PROJECT PROTOCOL

TERATOGENIC POTENTIAL OF CAFFEINE IN OSBORNE-MENDEL RATS (ADMINISTERED VIA WATER BOTTLES)

Objective: Caffeine, a naturally occurring substance and a food additive when added to soft drinks, is one of the compounds which is known to cross the placental barrier. It has been studied in numerous teratology and reproduction studies utilizing various modes of administration, numerous species and strains of animals, and many regimens. A full review of these articles is found in a memorandum dated August 8, 1978. Due to deficiencies that exist within these various studies and due to the fact that many were conducted in the 1960's and early 1970's, it was thought that studies should be run to attempt to elucidate whether caffeine is a teratogen and if so, to further resolve the no-effect level. These protocols will not deal with the teratological studies done by intraperitoneal and subcutaneous dosing, since they are of little relevance to the Bureau of Foods and have very limited usage, nor will they address the question of dietary feed since little caffeine is consumed in food.

Proposed Starting Date: Depending upon supply of animals and availability of adequate personnel and equipment.

Proposed Ending Date: Animals will be treated 20 days during pregnancy but it is impossible to determine how quickly the animals will mate.

Name of Study Director: Thomas F.X. Collins, Ph.D.

Other Principal Investigators Involved: Thomas Black, M.S., John Welsh, Ph.D.

Technicians Involved: George Gray, John Goodman, James Rorie.

Animal Care Personnel: John Goodman, James Rorie, Michael Scott

Skeletons Read By: John Welsh, Ph.D.

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Testing Facility: Mammalian Reproduction and Teratology Unit
Whole Animal Toxicology Branch
Division of Toxicology
FDA.

Room to be Utilized: Rooms 5452 and 5454

Compound Information: Caffeine, the alkaloid 1,3,7-trimethylxanthine, was purchased from the Eastman Kodak Company (lot B7X) and has been submitted to the Analytical Chemistry and Physics Branch for a thorough chemical analysis and stability determination. Preliminary indications are that the samples are approximately 99% pure.

The estimated caffeine consumed from cola beverages over a 14-day period has been shown to vary from 5.5 to 16.0 mg/kg/day. In humans, caffeine appears to equilibrate freely between plasma and tissue water; it has been shown to cross the placental barrier. Ingested caffeine is absorbed, metabolized and excreted in the urine as xanthine derivatives.

Animals and Animal Husbandry Procedures: Osborne-Mendel rats (FDA strain) are obtained from the FDA breeding colony. The females are between 100 and 140 days of age and weigh between 220 and 270 grams when placed on the experiment. The males are sexually mature animals approximately 5-6 months of age.
144 males are utilized as sires in this experiment. Fifty pregnant females per dose level are utilized.

The animals are housed in hanging cages. The animals are mated in double hanging cages and the females are housed in single hanging cages. Animals are provided with ground Purina Chow. The animal pans are changed every Monday, Wednesday, and Friday except the mating racks which are changed daily to aid in the identification of vaginal plugs. Floors are mopped daily and there are at least two checks daily for morbid animals. The experimental solution is prepared and used within 24 hours.

Since the animals are given the compound over the entire period of pregnancy, this necessitates dosing to occur on weekends. Appropriate professional and technical staff are available to work during weekend work periods. The animals are uniquely identified by metal ear tags.

Methodology: The compound will be given in water bottles at dose levels of 0, 0.005, 0.009, 0.018, 0.036, 0.07, and 0.1%; this is approximately equivalent to 0, 6, 13, 25.9, 50.8, 100.8, and 144 mg/kg/day. The compound is dissolved in distilled water. The control animals are given distilled water. The compound is weighed on an analytical balance which is calibrated before each weighing by utilizing the scale calibration form (F-10) and the standard operating procedure for the calibration form (SOP-10). The solutions are made up in clean volumetric flasks.

The mating procedure is described in SOP-1 and records of all animals mated are kept on mating forms (F-1). Each evening at approximately 4:30 pm, 72 females which meet the requirements (i.e., weight

between 220 and 270 g and 100-140 days of age) are mated with 36 males which are randomly chosen from the male population available. The following morning, the females are removed, the females are individually smeared for the determination of sperm in the vaginal fluid. This is examined under a binocular microscope. The females exhibiting a positive sign of pregnancy are randomly (utilizing a table of random numbers) placed on the experiment. The date of finding sperm is considered day 0 of pregnancy. Each female is placed in an individual cage and given a known amount of ground chow and a water bottle containing distilled water or the appropriate amount of compound in solution. Each water bottle contains a sipper tube with a metal ball to prevent extraneous loss of fluid. The amount of food given and consumed is recorded on the food consumption form (F-5) and the amount of fluid consumed is recorded on the fluid consumption form (F-6). All data pertaining to experimental assignment are recorded on the appropriate form (F-2) according to the standard operating procedure for this form (SOP-2). The mating procedure continues until there are 50 females per dose level.

The animals and water bottles are weighed daily and these weights are recorded on the fluid consumption form (F-6). The food consumption and the weight of the females are also recorded on the food consumption form on days 7 and 14, and before cesarean section on day 20.

On day 20, the animals are sacrificed by carbon dioxide asphyxiation and a cesarean section is performed according to the procedures described in SOP-4. The following parameters are noted. The number and position of resorption sites and fetuses (dead or alive) are recorded, as well as corpora lutea data and number of implantation sites. Fetuses are examined individually. Fetus sex and number of normal and abnormal fetuses are recorded. Records of cesarean section data are made on forms F-4. Color photographs are taken on viable fetuses of the negative control or test substance group exhibiting grossly apparent anomalies. One-half of the fetuses, randomly selected, are submitted to visceral examination by Wilson technique and the remaining half of the fetuses are submitted to skeletal examination by Alizarin technique. The randomization, however, is not done on fetuses with grossly apparent anomalies; these are assigned to that method which will better define the anomaly.

The skeletons are stained according to the technique described in SOP-11 and the documentation of the staining is recorded on the appropriate stain documentation form (F-11). The skeletal analysis is based on procedures described in SOP-8 and also from skeletal atlases available in the laboratory.

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Data Reporting: Data reported will include, but not be limited to, the following elements:

Chemical tested (or control)
Species identification
Dose level
Dam identification
Dam weights daily
Food consumption (at 0, 7, 14, and 20 days)
Fluid consumption daily
Fate of dam (term, died, aborted) and date
Date dam sectioned
Number and positions of implantations
Number and positions of resorptions
Number, weight, length, and position of live fetuses
Number, weight, and position of dead fetuses
Pup identification
Abnormalities for each pup
Sex of each pup

Analysis of Data: All the data will be doubly validated and sent to the FDA's Division of Mathematics for statistical analysis. The specific analyses performed will be reported in the manuscript prepared from the data.

The data analysis methods are usually similar to those described in Food and Cosmetic Toxicology 10: 619-624, but may vary slightly depending on the latest information available on specific tests.

Measurement of Amount Consumed: The measurement of the amount of caffeine consumed will be done on a daily basis and can be calculated on a mg/kg basis for each animal and for each day of pregnancy. From previous studies done with this strain of animals, we have found that the Osborne-Mendel rat consumes approximately 36 ml of water per day. Based on this assumption, the dose levels administered are approximately 6, 13, 25.9, 50.8, and 100.8 mg/kg/day.

Sample calculation: Fluid consumed: 36 ml/day
Concentration: 0.009%
Animal weight: 250 g

$$36 \times .00009 = 3.24 \text{ mg}$$

$$\frac{3.24 \text{ mg}}{250 \text{ g}} = \frac{X}{1000 \text{ g}} \quad = 12.96 \text{ mg/kg/day}$$

Thomas F.X. Collins 1/4/79
Thomas F.X. Collins Date
Study Director

Frank Vocci — Date
Chief, Whole Animal Toxicology Branch

W. Gary Flamm — 12/2/79 — Date
Associate Director for Regulatory Evaluation

Eugene Sporn — 1/4/79 — Date
Associate Director for Laboratory Investigations

James Winbush — 12/27/78 — Date
Chief, Division of Mathematics

Protocol No.: 1151

January 12, 1979

FDA PROJECT PROTOCOL

TERATOGENIC POTENTIAL OF CAFFEINE IN OSBORNE-MENDEL RATS (ADMINISTERED VIA WATER BOTTLES)
ADDENDUM NO. 1

Compound Information: Caffeine (Eastman Kodak Company lot B7X) has been tested by the Analytical Chemistry and Physics Branch and found to be stable in water for a period of 3-4 days. We have also placed caffeine in bottles in contact with the rats for the same time interval and have found that this has no influence on the stability of the caffeine. In addition, we have obtained information on studies done at Battelle Laboratories where caffeine appeared to be stable for 28 days in water at room temperature.

An analysis of lot B7X has been received from the Eastman Kodak Company and it appears to be at least 98% pure and greater than USP specifications.

Thomas F.X. Collins 1/12/79

Thomas F.X. Collins, Ph.D. Date
Study Director

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Daniel D. Jones
GRAS Review Branch, HFF-335

DATE: January 12, 1979

FROM : Mammalian Reproduction and Teratology Team
Division of Toxicology, HFF-155

SUBJECT: Caffeine Protocols

Attached are the protocols for the studies that will be initiated shortly on the teratogenic potential of caffeine in Osborne-Mendel rats (protocols No. 1150 and 1151). The protocols were based on our review of caffeine (memorandum of August 8, 1978).

Longer term studies will probably have to be undertaken to answer questions concerning the reproductive effects of caffeine. We have an additional protocol to study the reproductive and teratogenic effects of caffeine over several generations. This protocol is not attached because it has been decided by the Protocol Committee of the Division of Toxicology not to approve this protocol until the results of the teratology studies are in.

Thomas F.X. Collins, Ph.D.
Leader, Mammalian Reproduction
and Teratology Team

Concur:

Frank Vocci
Chief, Whole Animal Toxicology Branch

Eugene M. Sporn, Ph.D.
Associate Director for Laboratory Investigations

cc: S.A. Miller, HFF-1
A.C. Kolbye, HFF-100
M.S. Walton, HFF-165
S.H. Frazier, HFF-8